Anti-Tumor Effect of Curcumin on Human Cervical Carcinoma HeLa Cells *In Vitro* and *In Vivo*

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CLC number: R737.33 Document code: A Article ID: 1000-9604(2007)01-0032-05
10.1007/s11670-007-0032-6

ABSTRACT

Objective: To investigate the anti-tumor effect of curcumin on human cervical carcinoma HeLa cells *in vitro* and *in vivo*. **Methods:** (1) Human cervical carcinoma cell line HeLa was cultured in *vitro*. HeLa cells were treated with 5-50 μmol/L curcumin for 24. 48, 72 h and the growth inhibition rates of HeLa cells were measured by MTT method. Cell apoptosis was inspected by electron microscopy and flow cytometry (FCM). (2) A transplanted tumor model by injecting HeLa cells into subcutaneous tissue of BABL/C mice was established and its growth curve was measured. 30 BABL/C mice with tumors were divided into 2 groups at random and 0.2 ml saline or 0.2 ml 250 μmol/L curcumin was injected into abdominal cavity respectively once everyday and lasted for ten days. The changes of tumor volume were measured continuously and tumor inhibition rate was calculated. At last the expressions of caspase-3 and bax protein in transplanted tumors were detected by immunohistochemistry. **Results**: (1) Curcumin inhibited the proliferation of Lela cells on a dose-depending manner. Apoptosis of cells could be observed by FCM. Partial cells presented the characteristic morphological changes of apoptosis under electron microseope. (2) When 1×10^7 HeLa cells were inoculated for each mouse, 100% of the mice developed growing tumors after seven days. An inhibition effect was observed in treatment group, and the inhibition rate of curcumin was 74.33%. The expressions of caspase-3 and bax in the transplanted tumors were increased in curcumin group. **Conclusion**: Curcumin is effective as an anti-cancer drug not only in *vitro* but also *in vivo*.

Key words: **Curcumin; Cervix neoplasm; Apoptosis; Immunohistochemistry; Flow cytometry**

Curcumin, a deferuloymethane, is a major active component of the food flavor turmeric (Curcuma Longa). Because of its stable colour and luster and low toxicity, curcumin has been widely used as food additive and coloring agent. Recently, it has been reported to possess anti-inflammatory, antioxidation and antiviral activities. Now, attention has been focused on its antitumor activity[1, 2]. *In vitro* curcumin was found to induce apoptosis of a wide variety of tumor cells including mice sarcoma S180 cells, human colon carcinoma HT-29 cells, human renal carcinoma 293 cells, human liver carcinoma HepG2 cells etc[3]. In our previous study, we have some primary results in the effect of curcumin on human cervical carcinoma[4]. In this study, we further investigated the anti-tumor effect of curcumin on HeLa cells, both *in vitro* and *in vivo*.

Received: Dec. 23, 2006; **Accepted :** Jan. 26, 2007
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MATERIALS AND METHODS

Materials

HeLa cell line was supplied by the department of pathophysiology of Chongqing Medical University. Curcumin and 3-(4, 5-demethy-2-thiazolyl)-2, 5-dephenyl-2h-tetrazolium-bromid (MTT) were obtained from Sigma (St Louis, Mo, USA). RPMI-1640 and fetal bovine serum (FBS) were from Hyclone. The mouse monoclonal anti-Bax and anti-Caspase-3 antibodies were purchased from Fuzhou Maixin Biotechnology Co. (China).

MTT Assay

Cell growth was measured by a modified MTT assay. About 1×10^5 cells/well were plated in 96-well

microplates and incubated overnight. Cells were then treated with 5, 10, 25 and 50 μmol/L curcumin for 24, 48 and 72 h. Then 20 μl stock MTT was added to each well and the cells were further incubated at 37°C for 4 h. The supernatant was removed and 200 μl DMSO in isopropanol was added to each well to solubilize the formazan product. The absorbance at wavelength of 570 nm was measured by a micro ELISA reader (Sigma). The negative control well contained medium only. The ratios of the absorbances of treated cells relative to those of the control wells were calculated and expressed as percentage of growth inhibition.

Flow Cytometry

The induction of apoptosis was analyzed by using the Annexin V-PE Apoptosis Detection Kit I according to the manufacturer's instructions[5]. Briefly, cells were inoculated at a cell density of 1.0×10^5 cells/ml, treated with 5, 10, 25 and 50 μmol/L curcumin and cultured for 48 h. After cultivation, these cells were washed twice with ice-cold phosphate-buffered saline (PBS) and then resuspended in a binding buffer [10 mmol/l HEPES/NaOH (pH 7.4), 140 mmol/L NaCl, 2.5 mmol/L $CaCl_2$] at a cell density of 1.0×10^6 cells/ml. The cell suspension (100 μl) was transferred to a 5-ml culture tube to which 5 μl of annexin V-PE and 5 μl of 7-AAD were added as fluorescent dyes. After incubation for 15 min at 25°C in the dark, 400 μl of binding buffer was added to the solution. Flow cytometry analysis was performed within 1 h according to the manufacturer's instructions.

Electron Microscopy

Cells were planted in 50 ml plastic culture flask and incubated in the absence or presence of 25 μmol/L curcumin at 37°C in 5% CO_2 for 48 h. Cells were collected into 1.5 ml Ep tubes and fixed with 2.5% paraformaldehyde for 5 min. Sections were prepared and photographed under Hitach 600 electron microscopy (Japan).

Animal Experiment

Five-week-old female normal BALB/c mice, purchased from our university, were used in this study and maintained under extremely clean conditions. To establish tumors in the BALB/c mice, HeLa cells were subcutaneously injected into the mid-dorsal region of the mice at a cell density of 1×10^7 cells suspended in 100 μl of PBS. Tumors were allowed to grow until the tumor volume was increased to approximately 0.2 cm^3. After tumor growth, the mice were injected with 250 μmol/L of curcumin in 200 μl PBS or the same volume of PBS via abdominal cavity once everyday and lasted for ten days. The changes of tumor volume were measured continuously and tumor inhibition rate of curcumin was calculated. Tumor inhibition rate (IR)=(average weight of control group-average weight of curcumin group)/average weight of control group×100%. IR<30 was considered ineffective, while IR≥30 was considered effective[6, 7].

Immunohistochemistry

The tumor tissues were fixed with 10% paraformaldehyde at room temperature for 24 h. The paraffin-embedded specimens were cut into sections with a thickness of 5 μm. The detection procedure was done as described in Kit protocol[8]. PBS instead of the first antibodies was used in the negative control. The Bax and Caspase-3 positive cells were defined when there was an aggregation of brown particles in the cytoplasm of the tumor cells. And the rate of the positive expressions = (the positive cells/500 tumor cells) × 100%.

Statistical Analysis

The data were mean values of at least three different experiments and expressed as $\bar{x}\pm s$. The student's *t*-test was used to compare data. $P<0.058$ was considered to be statistically significant.

RESULTS

Effects of Curcumin on Cell Proliferation

The effects of curcumin on growth of HeLa cells are shown in Fig. 1. It was showed that cells of control group growth actively. Compared with control group, the growth of cells treated with different concentrations of curcumin was inhibited significantly in a concentration and time-dependent manner. After 72 h, inhibition rate was in the range of 11%-45.8%. Statistical analysis showed that the differences between samples with different treatment times were very significant ($P<0.01$). Compared with the control group, the differences of the groups of 5 and 10 μmol/L were significant ($P<0.05$) and between the groups of 25 μmol/L and 50 μmol/L were very significant ($P<0.01$).

Apoptosis of HeLa Cells Induced by Curcumin

Flow cytometry analysis of the apoptosis induced by curcumin was carried out by performing annexin